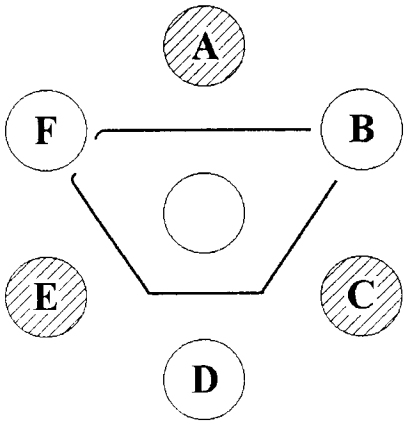


FIGURE 3.—*Immunodiffusion test that has AI AGID antigen in the center well; AI-positive control serum in wells A, C, and E; AI-negative test serum in well B; AI-positive test serum in well D; and weak positive test serum in well F.*

(b) The enzyme-linked immunosorbent assay (ELISA) may be used as a screening test for avian influenza. Use only federally licensed ELISA kits and follow the manufacturer's instructions. All ELISA-positive serum samples must be confirmed with the AGID test conducted in accordance with paragraph (a) of this section.

[65 FR 8019, Feb. 17, 2000]

Subpart B—Bacteriological Examination Procedure

§ 147.10 Laboratory procedure recommended for the bacteriological examination of egg-type breeding flocks with salmonella enteritidis positive environments.

Birds selected for bacteriological examination from egg-type breeding flocks positive for *Salmonella enteritidis* after environmental monitoring should be examined as described in § 147.11(a) of this subpart, with the following exceptions and modifications allowed due to the high number of birds required for examination:

(a) Except when visibly pathological tissues are present, direct culture, § 147.11(a)(1) of this subpart, may be omitted; and

(b) Enrichment culture of organ (non-intestinal) tissues using a non- selective broth, § 147.11(a)(2) of this subpart, may be omitted.

[59 FR 12801, Mar. 18, 1994]

§ 147.11 Laboratory procedure recommended for the bacteriological examination of salmonella.

(a) *For egg- and meat-type chickens, waterfowl, exhibition poultry, and game birds.* All reactors to the Pullorum-Typhoid tests, up to 25 birds, and birds from *Salmonella enteritidis* (SE) positive environments should be cultured in accordance with both the direct (paragraph (a)(1)) and selective enrichment (paragraph (a)(2)) procedures described in this section. Careful aseptic technique should be used when collecting all tissue samples.

(1) Direct culture (refer to illustration 1). Grossly normal or diseased liver, heart, pericardial sac, spleen, lung, kidney, peritoneum, gallbladder, oviduct, misshapen ova or testes, inflamed or unabsorbed yolk sac, and other visibly pathological tissues where purulent, necrotic, or proliferative lesions are seen (including cysts, abscesses, hypopyon, and inflamed serosal surfaces) should be sampled for direct culture using either flamed wire loops or sterile swabs. Since some strains may not dependably survive and grow in certain selective media, inoculate non-selective plates (such as blood or nutrient agar) and selective plates (such as MacConkey [MAC] and brilliant green novobiocin [BGN] for pullorum-typhoid and MAC, BGN, and xylose-lysine-tergitol 4 [XLT 4] for SE). After inoculating the plates, pool the swabs from the various organs into a tube of non-selective broth (such as nutrient or brain-heart infusion). Refer to illustration 1 for recommended bacteriological recovery and identification procedures.[7] Proceed immediately with collection of organs and tissues for selective enrichment culture.

(2) Selective enrichment culture (refer to illustration 1). Collect and culture organ samples separately from intestinal samples, with intestinal tissues collected last to prevent cross-contamination. Samples from the following organs or sites should be collected for culture in selective enrichment broth:

(i) Heart (apex, pericardial sac, and contents if present);

(ii) Liver (portions exhibiting lesions or, in grossly normal organs, the drained gallbladder and adjacent liver tissues);

(iii) Ovary-Testes (entire inactive ovary or testes, but if ovary is active, include any atypical ova);

(iv) Oviduct (if active, include any debris and dehydrated ova);

(v) Kidneys and spleen; and

(vi) Other visibly pathological sites where purulent, necrotic, or proliferative lesions are seen.

(3) From each bird, aseptically collect 10 to 15 grams of each organ or site listed in paragraph (a)(2) of this section. Mince, grind, or blend and place in a sterile plastic bag. All the organs or sites listed in paragraph (a)(2) of this section from the same bird may be pooled into one bag. Do not pool samples from more than one bird. Add sufficient tetrathionate enrichment broth to give a 1:10 (sample to enrichment) ratio. Follow the procedure outlined in illustration 1 for the isolation and identification of *Salmonella*.

(4) From each bird, aseptically collect 10 to 15 grams of each of the following parts of the digestive tract: Crop wall, duodenum, jejunum (including remnant of yolk sac), both ceca, cecal tonsils, and rectum-cloaca. Mince, grind, or blend tissues and pool them into a sterile plastic bag. Do not pool tissues from different birds into the same sample. Add sufficient tetrathionate enrichment broth to give a 1:10 (sample to enrichment) ratio. Follow the procedure outlined in illustration 1 for the isolation and identification of *Salmonella*.

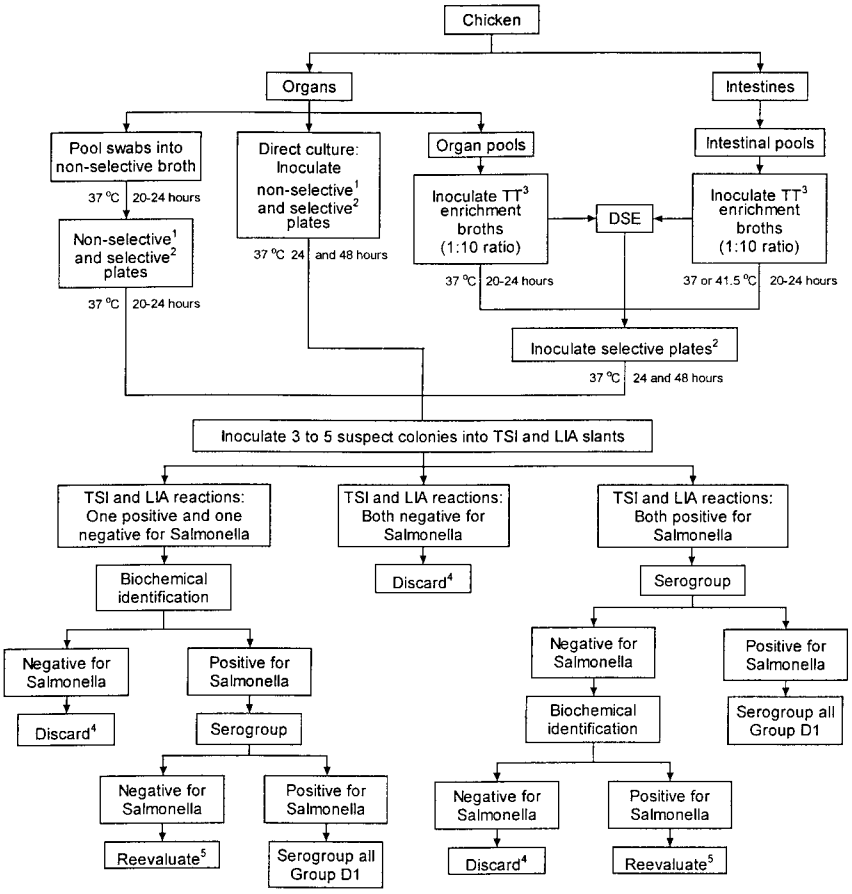
(5) After selective enrichment, inoculate selective plates (such as MAC and BGN for pullorum-typhoid and MAC, BGN, and XLT 4) for SE. Inoculate three to five *Salmonella*-suspect colonies from plates into triple sugar iron (TSI) and lysine iron agar (LIA) slants. Screen colonies by serological (i.e., serogroup) and biochemical procedures (e.g., the Analytical Profile Index for Enterobacteriaceae [API]) as shown in illustration 1. As a supplement to screening three to five *Salmonella*-suspect colonies on TSI and LIA slants, a group D colony lift assay may be utilized to signal the presence of hard-to-detect group D *Salmonella* colonies on agar plates.

(6) If the initial selective enrichment is negative for *Salmonella*, a delayed secondary enrichment (DSE) procedure is used. Leave the tetrathionate-enriched sample at room temperature for 5 to 7 days. Transfer 1 mL of the culture into 10 mL of fresh tetrathionate enrichment broth, incubate at 37 C for 20 to 24 hours, and plate as before.

[7] Biochemical identification charts may be obtained from ''A Laboratory Manual for the Isolation and Identification of Avian Pathogens,'' chapter 2, Salmonellosis. Fourth edition, 1998, American Association of Avian Pathologists, Inc., Kennett Square, PA 19348.

(7) Serogroup all isolates identified as salmonellae and serotype all serogroup D1 isolates. Phage-type all SE isolates.

Illustration 1.—Procedure for culturing Pullorum-Typhoid reactors and birds from SE-positive environments.



1. Non-selective plates such as blood or nutrient agar.
2. Selective plates such as MacConkey, Brilliant Green Novobiocin (BGN) for pullorum-typhoid reactors and MacConkey, BGN, and xylose-lysine tergitol 4 (XLT 4) for SE.
3. Tetrathionate enrichment broth.
4. Reevaluate if epidemiologic, necropsy, or other information indicates the presence of an unusual strain of Salmonella.
5. If biochemical identification and serogroup procedures are inconclusive, restreak original colony onto non-selective plating media to check for purity. Repeat biochemical and serology tests.

(b) *For turkeys*—(1) *Bacteriological examination of Salmonella reactors and necropsy specimens.* Grossly normal or diseased liver, heart, pericardial sac, spleen, lung, kidney, pancreas, peritoneum, drained gallbladder, oviduct,

misshapen ova, testes, inflamed or unabsorbed yolk sac, and other visibly pathological tissues where purulent, necrotic, or proliferative lesions are seen (including cysts, abscesses, hypopyon, and inflamed serosal surfaces), should be directly cultured by means of a flamed wire loop or with sterile swabs.[8] Careful aseptic technique must be utilized throughout the process of collecting tissues. Selective media should not be relied upon to deal with contaminants, since some strains may not dependably survive and grow in certain selective media. Inoculate veal infusion (VI) and brilliant green (BG) agar plates. Incubate the plates for 24 and 48 hours at 37°C. The digestive system should always be cultured separately (see paragraph (b)(7) of this section) after other anatomical organs and systems have been collected and cultured.

(2) *Bacteriologic examination of environmental and other contaminated specimens.* (i) Culture a representative sample of the specimen in tetrathionate Hajna (TTH) selective broth (TT Mueller-Kauffmann or selenite-cystine is also acceptable) as a temperature of 41–42 °C for 24 hours. Note: Do not use selenite-cystine if double strength skim milk is used as a preservative for the sample.

(ii) Inoculate an agar late of brilliant green novobiocin (BGN) and an agar plate of xylose-lysine-tergitol 4 (XLT4), incubate at 37 °C for 24 hours, and retain culture tubes at room temperature for 5–7 days for possible reculturing of the negative tubes using 0.25 ml in TTH.

(iii) Inoculate *Salmonella* suspect colonies to slants of triple sugar-iron (TSI) and lysine-iron (LI) agar and incubate at 37 °C for 24 hours. Five colony picks per plate should be taken unless 50 percent or more of the plates have *Salmonella*- like colonies. In that case, the number of picks may be reduced to three per plate. A group D colony lift assay may be utilized to signal the presence of the hard-to-detect group D salmonella colonies on agar culture plates.

(iv) Conduct serologic screening of cultures revealing typical reactions of *Salmonella* on TSI and LI agar slants using somatic O-group antisera agglutination or transfer for further identification to appropriate biochemical tests such as: Dextrose, lactose, sucrose, mannitol, maltose, dulcitol, malonate, gelatin, urea broth, citrate, lysine decarboxylase, ornithine decarboxylase, methyl red and Voges-Proskauer, KCN, salicin broths, indole, and hydrogen sulfide. Motility or nonmotility is demonstrated by inoculating a suitable semisolid medium. The Analytical Profile Index API 20E)[9] for Enterobacteriacea (APE) system may also be used for further identification if desired.

(v) Serotype all *Salmonella* group D cultures.

(3) The following organs should be aseptically collected for culture:

(i) Heart (apex, pericardial sac, and contents if present.);

(ii) Liver (portions exhibiting lesions or, in grossly normal organs, the drained gallbladder and adjacent liver tissues.);

(iii) Ovary-Testes (entire inactive ovary or testes, but if ovary is active, use own judgment and include any atypical ova.);

(iv) Oviduct (if active, include any debris and dehydrated ova.);

(v) Pancreas and kidneys; and

(vi) Spleen.

(4) Aseptically collect 10–15 g or whatever lesser amount is available of each organ or site listed in paragraph (b)(3) of this section from each reactor, and grind or blend them completely in 10 times their volume of VI broth. Organs may be processed individually or in combinations where appropriate. Suspensions should be transferred in 10-ml aliquots to 100-ml of both VI and tetrathionate brilliant green (TBG) broth and incubated at 37 °C for 24 hours. Plate the VI broth on VI and BG

[8] Culture media preparation and biochemical identification charts can be obtained from *Culture Methods for the Detection of Animal Salmonellosis and Arizonosis,* Committee on Salmonellosis and Arizonosis, AAVLD, 1976 Iowa State University Press, Ames, IA 50010.

[9] We use trade names solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement over other products not mentioned.

agar and plate the TBG broth on BG agar and incubate at 37 °C. Examine these plates after 24 and 48 hours of incubation. The contents of the gallbladder can be cultured separately by inoculating 10-ml of VI and TBG broth with cotton swabs and incubating at 37 °C for 24 hours. Plate on BG agar and incubate at 37 °C. Examine these plates after 24 and 48 hours of incubation. If contamination with pseudomonas or proteus is a problem, make platings on BG sulfapyridine (BGS) agar.

(5) Where field samples are directly inoculated into enrichment broths and a delay of several days occurs before they reach a laboratory, or if recovery of low numbers or organisms is expected from a primary culture, a secondary enrichment culture should be prepared. Subculture a week-old primary culture by transferring 1-ml of inoculum into a fresh tube 10-ml of enrichment broth. This secondary enrichment should be incubated at 37 °C for 24 hours before plating. (See paragraph (b)(1) of this section.) TBG broth is recommended for this procedure.

(6) Make a composite sample of the following parts of grossly normal or diseased tissues from the digestive tract: Crop wall, duodenum, jejunum (including remnant of yolk-sac attachment), both ceca, cecal tonsils, and rectum-cloaca. Aseptically collect 10–15 g of each organ or tissue, or whatever lesser amount is available, and grind or blend them completely in 10 times their volume of TBG broth. Transfer 10-ml of a composite sample of a suspension from the digestive tract into 100-ml of TBG broth, and incubate flasks at 42 °C for 24 hours. Cultures may be incubated at 37 °C if 42 °C incubators are not available. The higher incubation temperatures for TBG broth reduce populations of competitive contaminants common in gut tissue. Plate on BG agar and incubate at 37 °C. Examine the plates after 24 and 48 hours of incubation. If contamination with pseudomonas or proteus is a problem make platings on BGS agar.

(7) If preferred, individual cotton swab samples may also be taken from the upper, middle, and lower intestinal tract (including both ceca and the rectum-cloaca). Deposit swabs in 10-ml of TBG broth and incubate and plate as described in paragraph (b)(6) of this section.

(8) Transfer suspect colonies to triple-sugar-iron (TSI) agar and lysine-iron (LI) agar and incubate at 37 °C for 24 hours.

(9) Cultures revealing typical reactions of salmonellae on TSI and LI agar slants should be transferred to any of the following appropriate biochemical tests for final identification: Dextrose, lactose, sucrose, mannitol maltose, dulcitol, malonate, gelatin, urea broth, citrate, lysine decarboxylase, ornithine decarboxylase, methyl red and Voges-Proskauer, KCN, salicin broths, indole, and hydrogen sulfide. Motility or nonmotility is demonstrated by inoculating a suitable semisolid medium.[10] The Analytical Profile Index for Enterobacteriaceae (API) system may be utilized for identification if feasible. For arizonae identification, make readings daily up to 10 days. An O-nitrophenyl-beta-D-galactopyranside (ONPG) disc may be used to identify slow lactose fermenters.[11]

(10) All salmonella cultures should be serologically typed.

(Approved by the Office of Management and Budget under control number 0579–0007)

[36 FR 23121, Dec. 3, 1971. Redesignated at 44 FR 61586, Oct. 26, 1979, and amended at 47 FR 21994, May 20, 1982; 50 FR 19900, May 13, 1985; 57 FR 57342, Dec. 4, 1992; 59 FR 12801, Mar. 18, 1994; 61 FR 11521, Mar. 21, 1996; 63 FR 3, Jan. 2, 1998; 65 FR 8019, 8023, Feb. 17, 2000; 67 FR 8469, Feb. 25, 2002]

§ 147.12 Procedures for collection, isolation, and identification of Salmonella from environmental samples, cloacal swabs, chick box papers, and meconium samples.

Information concerning the pen arrangement and number of birds per pen should be obtained from the owner so that the required number of samples

[10] Formulation for the semisolid motility medium can be obtained from: *Isolation and Identification of Avian Pathogens,* American Association of Avian Pathologists, University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania 19348—1692, 1980.

[11] ONPG discs are available from: Baltimore Biological Laboratories, Cockeysville, MD 21030.